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REAGENT

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Technical field of the Invention

The present invention relates to a reagent and to a medical agent comprising said reagent, to a kit for treatment or diagnosing lymphoma, to use of said medical agent, and to a method for treatment of lymphoma.

Background Art

Lymphomas are malignant cell infiltrations of the lymphatic system. The lymph system includes the nodes which are located in the neck, armpit, and groin. These nodes are only part of the lymph system, as they are connected to each other and to the spleen, thymus, and parts of the tonsils, stomach, and small intestine by a network of vessels. The vessels carry a fluid called lymph which contains lymphocytes. Once a malignancy begins in one part of the lymph system, it often spreads throughout the rest of the lymph system before it is detected.

There are precise, internationally agreed criteria to define the stage of disease for each type of cancer. For lymphomas this means mapping out how many lymph nodes are affected. It also means finding out if the lymphoma has spread outside the lymphatic system to other organs.
Stage I: Cancer limited to one group of lymph nodes or a single organ or site outside the lymphatic system
Stage II: Cancer in two or more groups of lymph nodes all on the same side of the diaphragm
Stage III: Cancer on both sides of the diaphragm but not outside the lymphatic system
Stage IV: Widespread cancer outside the lymphatic system

Lymphomas, are divided into many sub-groups according to cell types. Generally, they are classified as either non-Hodgkin's and Hodgkin's. Currently, Hodgkin's lymphoma is more curable than non-Hodgkin's. Non-

Hodgkin's lymphomas are derived from both B-cells and T-cells origins, where 90% of all cases are B-cell derived and the remaining 10% are of T-cell derivation.

The treatment for all types of lymphoma depends on the type, stage, and grade of disease. The stages and grades are outlined below.

Stages:

I: cancer site, no bone marrow involvement

II: two sites, both either above or below the diaphragm; no bone marrow involvement

III: sites above and below the diaphragm; no bone marrow involvement

IV: bone marrow is affected or the cancer cells have spread outside the lymphatic system

Grades:

high: usually found in B-cell and T-cell types

intermediate: usually found in B-cell and T-cell types

low: predominantly found in B-cell types

Lymphomas are usually treated by a combination of chemotherapy, radiation, surgery, and/or bone marrow transplants. The cure rate varies greatly depending on the type of lymphoma and the progression of the disease.

Because lymph tissue is found in many parts of the body, non-Hodgkin's lymphoma can start in almost any part of the body. The cancer can spread to almost any organ or tissue in the body, including the liver, bone marrow, spleen, and nose.

Based on the histology, non-Hodgkin's lymphoma are divided into two groups: indolent lymphomas, which are slower growing and have fewer symptoms, and aggressive lymphomas, which grow more quickly.

Lymphomas include follicular small cleaved cell lymphoma, adult diffuse mixed cell lymphoma, follicular mixed cell lymphoma, adult diffuse large cell lymphoma,

follicular large cell lymphoma, adult immunoblastic large cell lymphoma, adult diffuse small cleaved cell lymphoma, adult lymphoblastic lymphoma, small lymphocytic (marginal zone) adult small non-cleaved cell lymphoma.

Other types of indolent non-Hodgkin's lymphoma/-leukemia are lymphoplasmacytoid lymphoma, monocytoid B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, splenic marginal zone lymphoma, hairy cell leukemia, and cutaneous T- cell lymphoma (Mycosis fungoides/Sezary syndrome).

Other types of aggressive non-Hodgkin's lymphoma are anaplastic large-cell lymphoma, adult T-cell lymphoma/-leukemia, mantle cell lymphoma, intravascular lymphomatosis, angioimmunoblastic T-cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, peripheral T- cell lymphoma, lymphoblastic lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary central nervous system lymphoma, and primary effusion lymphoma. Aggressive lymphomas are also seen more frequently in patients who are HIV-positive (AIDS-related lymphoma).

Recurrent adult non-Hodgkin's lymphoma may come back in the lymph system or in other parts of the body.

Indolent lymphoma may come back as aggressive lymphoma. Aggressive lymphoma may come back as indolent lymphoma.

Non-Hodgkin´s lymphomas (NHLs) are the fifth leading cause of cancer morbidity and mortality (Wingo P, Tong T, Bolden S. Cancer statistics, 1995. CA Cancer J. clin 1995; 45, 8-30 & Parker SL, Tong T, Bolden S., Wingo PA. Cancer statistics, 1996. CA Cancer J. Clin 1996;46:5-27). Over the past two decades, the prevalence in USA of these lymphomas has increased rapidly. Five years ago more than 52.000 new diagnosis were made, and 23.000 deaths were attributed to NHLs and with an incidence increasing with a rate of 7% per year (Parker SL, Tong T, Bolden S., Wingo PA. Cancer statistics, 1996. CA Cancer J. Clin

1996;46:5-27). This represents an increase of 150% in the population-adjusted new cases of NHLs over the past 50 years.

The overwhelming majority of patients (about 80%) constitute patients with NHLs of B-cell origin (Harris N.L., Jaffe E.S., Stein H. et.al.. Lymphoma classification proposal: clarification [letter]. Blood 1995; 85: 857-860). Despite the use of various combined chemotherapeutic regimens for advanced-stage intermediate- and high grade lymphomas, roughly half of patients treated do not have a complete remission or eventually have a relapse after a remission. The situation has not improved noticeably in almost two decades (Gordon LI, Harrington D, Andersen J, et.al. N. Engl. J. Med. 1992;327:1342-9 & Fisher RI, Gaynor ER, Dahlberg S, et.al. N. Engl. J. Med. 1993; 328: 1002-6).

Treatment with standard-dose salvage chemotherapy rarely results in durable remissions and often has serious toxicity. Although the use of high-dose chemotherapy with bone marrow transplantation has shown promise, not all patients derive long-term benefits from this type of treatment (Armitage JO. Blood 1989;73:1749-58). A curative treatment for patients with advanced low-grade lymphoma still remains to be clearly established (DeVite VT Jr., Jaffe ES, Mauch P, Longo DL. Lymphocytic lymphomas. In: DeVita VT Jr., Hellman S., Rosenberg SA. Eds. Cancer: principles and practice of oncology. 3rd ed. Vol.2. Philadelphia: J.B. Lippincott, 1989;1741-98). Treatment with anthracycline-based chemotherapy regimes results in complete remission in 50-90 percent of patients with intermediate and high-grade non-Hodgkin's lymphoma and long-term disease-free survival in 30 to 60 percent.

Unfortunately, few patients with low-grade lymphoma or relapses of any type of lymphoma can be cured with conventional approaches (Armitage JO. N.Engl.J.Med. 1993; 328:1023-30). High-dose chemoradiotherapy with bone

marrow transplantation cures 10-50% of patients with lymphoma in relapse, but 40-80 % relapse again and 5-20 percent die of complications related to transplantation (Appelbaum FR, Sullivan KM, Buckner CD et.al. J.Clin. Oncol. 1987;5:1340-7 & Freedman AS, Takvorian T, Anderson KC et.al. J.Clin.Oncol. 1990;8:784-91). The use of large doses of chemoradiotherapy has not been feasible because of unacceptable morbidity and mortality (Bearman SI, Appelbaum FR, Bruchner CD, et.al. J.Clin.Oncol. 1988; 6:1562-8).

Tissue or organ specific localisation of a medical agent is a very important factor in its effective application. Lack of specific tissue localisation is of particular importance in the treatment with cytotoxic agents, where the desired effect is to kill certain types of cells, such as in the treatment of cancer.

The treatment of cancer with agents specific for the tumour cell without harming of the host has long been a goal of oncology.. The development of monoclonal antibodies provided hope that tumour-targeted therapy would one day play a role in the treatment of cancer. Indeed, promising results have been presented in several areas; however, most of the treatment modalities have often proved technically difficult, produced disappointing efficacy, and were often not broadly applicable to patients with a given malignancy.

The treatment of patients with lymphoma is an exception. Patients with advanced stage or relapsed low-grade non-Hodgkin's lymphoma (NHL) are not curable using conventional approaches and are usually treated with combination chemotherapy regimens of increasing intensity as needed to reduce disease and palliate symptoms. Recent attempts utilising supralethal chemotherapy combined with radiotherapy followed by bone marrow transplantation have resulted in an approximately 20 % long term disease-free survival rate (F. Applebaum et al, J. Clin.Oncol. 5:1340, 1987). However, most patients treated in this manner die

of lymphoma or treatment complications. Therefore, new strategies for the treatment of non-Hodgkin's lymphomas are needed. These strategies should be aiming at the maximisation of therapeutic effect coupled with the minimization of toxicity.

One approach involves the use of monoclonal antibodies that recognise tumour-associated antigens as a means of targeting drugs or radioisotopes to tumour cells. This approach is particularly attractive in the case of NHL as the lymphoma tumour cells display a variety of tumour-restricted antigens on their cell surfaces that would be available for targeting (A.J. McMichael, Leukocyte Typing III, pp 302-363 and 432-469, Oxford University Press, Oxford, England, 1987). The rationale for utilising such an approach is further supported by the observation that monoclonal antibodies by themselves can exhibit anti-tumour effects in vivo. Of all the malignancies that have been treated with monoclonal antibodies to date, the lymphomas have yielded the most dramatic results. Significant tumour regressions have been reported in patients treated with monoclonal anti-idiotype antibodies (R.A Miller et, New Eng. J. Med. 306:517, 1982; T.C. Meeker et al, Blood 65:1349, 1985). Most of the tumour responses, however, have been incomplete and of relatively short duration. The practical problem of generating anti-idiotype antibodies restricts the utility of such an approach (T. Meeker et al, New. Eng. J. Med 312:1658, 1985).

Recently, a number of monoclonal antibodies have been developed which recognise antigenic sites on both malignant and normal human B cells. These pan B-cell antibodies have been useful in classifying lymphomas and in defining the ontogeny and biology of normal B cells. Because of the limited efficacy of unmodified antibodies in general, recent attention has focused on the use of antibodies conjugated to cytotoxic agents. Among the cytotoxic agents that might be considered, radioisotopes

are especially attractive, as lymphomas are especially sensitive to the effects of radiation. Moreover, such radiolabelled antibodies may be of considerable utility in terms of diagnostic imaging of tumour involved sites. Most of these cytotoxic anti-lymphoma antibodies are directed towards CD20.

CD20 is an antigen that is a 35 kilodaltons, non-glycoylated phosphoprotein found on the surface of greater than 90 % of B cells from peripherial blood or lymphoid organs. The antigen is expressed on the surface of virtually all resting B cells maintained in culture, but is lost by approximately one-third of the population upon activation of the cells by protein A or exposure to Epstein-Barr virus. This result has been interpreted to mean that CD20 is lost during terminal differentiation of B cells (L. M. Nadler, Lymphcyte typing II, vol 2 pp 3-37 and 65 Appendix, E. L. Renling et al eds Springer Verlag, 1986).

A number of other antigens like the CD19 are also expressed on the surface of cells of the B lineage. However, contrary to the CD20, antibodies binding to the CD19 are rapidly internelised. Other antibodies identified as binding to these types of cells are: the B2 binding to the CD21 antigen; B3 binding to the CD22 antigen and the J5 binding to the CD 10 antiden. The pan-B-cell antibody MB-1 is also of interest and has been shown to bind to CD37.

Naked antibodies directed against CD20 have shown to have efficacy. One registered naked antibody, Rituximab, is a chimeric mouse/human anti-CD20 antibody that has shown efficiency in the treatment of indolent lymphoma, especially follicular lymphoma. The overall response rate for patients with indolent lymphoma is 50 % and the complete response rate is 10% (McLaughlin P et al, J. Clin. Oncol. 16: 2825-2833, 1998.). Time to progression has been reported to be 13 months. Rituximab does also produce objective remissions in aggressive lymphoma albeit

with a lower response rate. Nonetheless virtually all patients treated with Rituximab as a single agent will eventually relapse.

Systemic radiotherapy is an established form of treatment. The use of radioiodine in the treatment of disseminated cancer of the thyroid is often the mainstay of therapy. Radioimmunotherapy (RIT) is another form of systemic radiotherapy where the radionuclide is targeted by an antibody to a tumour cell. RIT is in some cases a combined modality between radiotherapy and immunotherapy, since the antibody itself may exert an anti-tumour effect. The use of RIT is still experimental, but several encouraging studies have been published. In treatment of B-cell lymphoma several groups have reported long term remissions following RIT. Most investigators have used ^{131}I or ^{90}Y labelled mouse antibodies directed to the CD20 antigen (Kaminski, M. S. et al, J. Clin. Oncol., 14:1974 -1981, 1996, Knox, S. J et al, Clin. Cancer Res., 2: 457-470, 1996.).

Therapeutic application of chimeric and radiolabelled antibodies for treatment of B cell lymphoma are descried Anderson, D.R. et.al. in EP 0 752 248 B1; EP 669 836 B1 and US 5,843,439; 5,776,456; 5,736,137. Methods for the treatment of lymphoma by administration of a B cell-specific antibody are described in Kaminski, M.S. et.al. US 5,595,721; 6,015,542; 5,843,398; 6,090,365 and by Goldenberg, D.M. et.al. in US 6,183,744 B1. Other patents and patent applications related to the subject matter are US 6,399,061 B1, EP 1 005 870 A2, WO 98/42378, WO 99/57981, WO 00/09160, WO 00/27428, WO 00/27433, WO 01/34194 A1, WO 01/10462 A1, WO 01/10460 A1, WO 00/67795, WO 00/52473.

Rituximab is a chimeric mouse/human antibody that has been engineered from its mouse parental antibody, ibritumomab. When ibritumomab is labelled with ^{90}Y, it is entitled Zevalin™. Wiseman et.al. Critical reviews in Oncology/Hematology 39 (2001), 181-194, have reported

that Zevalin™ may be administered safely without prior dosimetry at an activity of 15 MBq/kg to patients with a platelet count of > 149 x 10^9 /L. For patients with platelet counts of 100-149 x 10^9, an activity of 11.1 MBq/ kg is well tolerated. A prospective randomised trial of Zevalin in patients with relapsed or refractory indolent or transformed lymphoma compared to a standard course of Rituximab has been reported. Among 143 patients studied an overall response of 80% was found for the Zevalin group vs. 56 % in the group who received unlabelled Rituximab (P=0.002) and with 30% complete remission with Zevalin vs 16% CR for Rituximab (P=0.04) Zevalin has also been evaluated in patients with follicular lymphoma refractory to Rituximab. The response duration was significantly longer (8.4+ *vs.* 4 months) for Zevalin as compared with prior Rituximab (P=0.008).

Normal organ toxicity limits the amount of activity that safely can be administered to patients and thereby the absorbed dose to tumour. The first dose-limiting organ is the bone marrow. Localised B-cell lymphoma may be cured by external beam radiotherapy with a dose of 30 to 44 Gy. The dose that may be achieved with conventional radioimmunotherapy without the use of stem cell support is substantially lower. Wiseman et al has reported a median dose of 15 Gy in B-cell lymphoma in a phase III trial (Wiseman G et al., Critical reviews in Oncology/-Hematology 39 (2001) 181-194). The response rate was 80% objective response and 34 % complete response. The Seattle group using stem cell support has reported the highest remission rate 80% complete remissions (Liu Steven Y. et al., J. Clin. Oncol.16(10): 3270-3278, 1998). They estimated tumour sites to achieve 27 to 92 Gy.

The non-haematological dose-limiting toxicity was reversible pulmonary insufficiency, which occurred at doses $\geq$ 27 Gy to the lungs. Although the studies are not quite comparable they indicate a dose effect relation-

ship in RIT. If there is a dose relationship, it may be possible to increase efficacy if a higher dose to the tumour can be delivered. This may be most clinically relevant, since complete remission following RIT has been associated with longer duration of remission (Wahl et al., J.Nucl. Med.39:21S-26S, 1998.).

An obstacle to this is the radio sensitivity of the bone marrow. A higher absorbed dose to the bone marrow may cause myeloablation. Thus, the dose necessary to reach a more effective therapy is hampered by the accumulation of radioactivity in the blood circulation, leading to toxicity of normal organs, as bone marrow. Various means to clear blood from cytotoxic targeting biomolecules (e.g. therapeutic or diagnostic monoclonal antibodies) after intravenous administration have been reported (See review article by Schriber G.J. and Kerr D. E., Current Medical Chemistry 2:616-629, (1995)).

In the so-called avidin chase modality, avidin or streptavidin is administered systemically after administration of the therapeutic or diagnostic antibody to which biotin has been attached, at a time when a sufficient amount of the antibody has been accumulated in the tumour. Avidin or streptavidin will associate with the antibodies and the so formed immunocomplex will clear from the blood circulation via the reticuloendothelial system(RES) and be cleared from the patient via the liver. These procedures will improve the clearance of biotinylated cytotoxic antibodies. An alternative approach to the same end, is the use of anti-idiotypic antibodies. However, all these methods rely on the liver or kidney for blood clearance and thereby expose either or both of these vital organs as well as the urinary bladder to high dose of cytotoxicity.

Another major drawback of the methods is the immunogenicity of these agents, particularly the streptavidin, which prevent repetitive treatments once the immune response has been developed. Extracorporeal tech-

niques for blood clearance are widely used in kidney dialysis, where toxic materials build up in the blood due to the lack of kidney function. Other medical applications, whereby an extracorporeal apparatus can be used include: removal of radioactive materials; removal of toxic levels of metals, removal of toxins produced from bacteria or viruses; removal of toxic levels of drugs, and removal of whole cells (e.g cancerous cells, specific haematopoietic cells - e.g. B, T, or NK cells) or removal of bacteria and viruses.

Various methods have been proposed to rapidly clear radiolabelled antibodies from blood circulation after the tumour has accumulated a sufficient quantity of immunoconjugate to obtain a diagnosis or therapy. Some of the methods employed involve enhancement of the body's own clearing mechanism through the formation of immune complexes. Enhanced blood clearance of radiolabelled antibodies can be obtained by using molecules that bind to the therapeutic antibody, such as other monoclonal antibodies directed towards the therapeutic antibody (Klibanov et al, J. Nucl. Med 29:1951-1956 (1988); Marshall et al, Br. J. Cancer 69: 502-507 (1994); Sharkey et al, Bioconjugate Chem. 8:595-604, (1997), avidin/streptavidin (Sinitsyn et al J. Nucl. Med. 30:66-69 (1989), Marshall et al Br. J. Cancer 71:18-24 (1995), or glycosyl containing compounds which are removed by receptors on liver cells (Ashwell and Morell Adv. Enzymol. 41:99-128 (1974). Still other methods involve removing the circulating immunoconjugates through extracorporeal methods (See review article by Schreiber G.J. and Kerr D.E., Current Medical Chemistry 2:616-629 (1995)).

The extracorporeal techniques used to clear a medical agent from blood circulation are particularly attractive because the toxic material is rapidly removed from the body.

Application of these methods in the context of immunotherapy have been previously described (Henry Chemical Abstract 18:565 (1991); Hofheinze D. et al Proc. Am. Assoc. Cancer Res. 28:391 (1987); Lear J. K. et al Antibody Immunoconj. Radiopharm. 4:509 (1991); Dienhart D. G. et al Antibody Immunoconj. Radiopharm. 7:225 (1991); DeNardo S.J. et al J. Nucl. Med 33:862-863 (1992); DeNardo G.L. et al J.Nucl.Med 34:1020-1027 (1993); DeNardo G. L. J. Nucl. Med 33:863-864 (1992); and US patent No. 5,474,772 (Method of treatment with medical agents).

To make the blood clearance more effective and to enable processing of whole blood, rather than blood plasma as the above methods refer to, the medical agents (e.g. tumour specific monoclonal antibody carrying cell killing agents or radio nuclides for tumour localization) have been biotinylated and cleared by an avidin-based adsorbent on a column matrix. A number of publications provide data showing that this technique is both efficient and practical for the clearance of biotinylated and radionuclide labelled tumour specific antibodies (Norrgren K. et al, Antibody Immunoconj. Radiopharm. 4:54 (1991), Norrgren K. et al J. Nucl. Med 34:448-454 (1993); Garkavij M. et al Acta Oncologica 53:309-312 (1996); Garkavij M. et al, J. Nucl. Med. 38:895-901 (1997)).

These techniques are also described in EP 0 567 514 and US 6,251,394. The device Mitradep®, developed and manufactured by Mitra Medical Technology AB, Lund, Sweden, is based on this technology. By using the avidin-coated filter in conjunction with biotin labelled therapeutic antibodies, the blood clearance technique can be applied equally well for chimeric or fully humanised antibodies. Experimental data reveal that during a three-hour adsorption procedure, more than 90 per cent of the circulating biotinylated antibodies can be removed by the

Mitradep® system (Clinical Investigator's Brochure - Mitradep®).

In order to be adsorbed to the extracorporeal filter, the monoclonal antibodies carrying the cytotoxic agent (e.g. radionuclide) need to be biotinylated (biotin binds irreversible to the avidin in the filter) prior to administration to the patient. The number of biotinyl moieties per IgG molecule is in the range of 3-6, typically 4.

A further development of this method with simultaneous labelling of biotin and radionuclides is described in a patent application by S. Wilbur and B.E.B. Sandberg PCT/ SE98/01345, disclosing a trifunctional reagent for the conjugation to a biomolecule.

This later method has a number of advantages over the consequtive labeling of radio nuclides and biotinylation and is particularly attractive in cases where the naked (non-chelated) antibody is supplied to the hospital, since both the chelating group and the biotinyl groups have to be conjugated to the antibody in addition to the radiolabelling step.

However, in most cases the same type of functions (ε-amino groups) on the antibodies are utilized for coupling of the chelating groups and the biotinyl groups, leading to a competition of the most accessible sites.

Chelation and /or biotinylation of an antibody results in a heterogenous preparation, if for example a chelated antibody is determined to have 3 chelates per antibody the preparation contains a mixture of antibodies with 1 chelate/antibody to 7 chelates/antibody. As the chelate and biotin are linked to the same moeties on the antibody, antibodies with a higher number of chelates might have lower number of biotin. It might also results in antibodies with high number of chelates having no biotin at all.

This means that statistically, a population of the antibodies carrying radionuclide but not biotin will

circulate in the blood, and those antibodies will not be removed by the Mitradep® filter.

To facilitate the labelling of the naked therapeutic or diagnostic antibody and to ensure that the ratio of biotin and the radiolabel is one to one, Mitra Medical Technology AB, Lund, Sweden has developed a series of novel water soluble structure (Tag-reagent; MitraTag™) containing the two types of functions, thereby enabling simultaneous and site specific conjugation of chelating groups (for radiolabelling) and the biotin groups.

The Tag-reagent labelled with the chelating group DOTA, is called MitraTag-1033.

The present invention encompasses a reagent having an anti-lymphoma antibody reactive moiety, a medical agent comprising the reagent conjugated to an anti-lymphoma antibody, and various methods for the treatment of lymphatic cancer, i.e. lymphoma, and NHL in particular.

Summary of the Invention

The object of the present invention is to solve the above discussed problem in connection with treatment of certain lymphoma diseases. This object is achieved by the present invention as specified below.

The present invention relates in one aspect to a reagent for conjugation to an anti-lymphoma antibody, wherein the reagent is a single molecule with at least three functional parts,

a) wherein a trifunctional cross-linking moiety is coupled to

b) an affinity ligand via a linker 1,

c) to an effector agent via a covalent bond, optionally via a linker 2,

d) and to a biomolecule reactive moiety, optionally via a linker 3, characterised in that, said biomolecule reactive moiety is an anti-lymphoma antibody reactive moiety being capable of forming a bond with an anti-lymphoma antibody, thereby forming a conjugate.

In another aspect the present invention relates to a medical agent comprising said reagent conjugated to said anti-lymphoma antibody via the anti-lymphoma antibody reactive moiety of said reagent.

In a further aspect the present invention relates to a composition comprising said medical agent.

In a further aspect the present invention relates to a kit for extracorporeal elimination or at least reduction of the concentration of a non-tissue-bound therapeutic or diagnostic medical agent as defined above in the plasma or whole blood of a mammalian host, wherein said medical agent previously has been introduced to a mammalian host and kept therein a certain time in order to be concentrated to the specific tissues or cells by being attached thereto, said host comprising

a) the anti-lymphoma antibody
b) the reagent or the medical agent, and
c) an extracorporeal device comprising an immobilised receptor on to which the affinity ligand adheres.

In a further aspect the present invention relates to use of said reagent or medical agent for treatment of lymphoma, preferably non-Hodgkin's lymphoma.

In still a further aspect the present invention relates to a method for treatment of lymphoma, preferably non-Hodgkin's lymphoma.

Further advantages and objects with the present invention will now be described in more detail, inter alia with reference to the accompanying drawings.

Brief Description of the Drawings

Fig. 1 shows depletion of 1033-rituximab conjugates during recirculation through a miniaturised Mitradep®.

Fig. 2 shows a flow cytometric assay of binding to the CD20 positive cell line Raji.

Fig. 3 shows binding of 1033-conjugates to a CD20+(SB) and a CD20-(HSB) cell line.

Fig. 4 shows competitive inhibition of ^{125}I-labelled rituximab binding to SB cells by cold rituximab and 1033-rituximab conjugates.

Fig. 5 shows whole body clearance of radioactivity in rats injected with ^{111}In-1033-rituximab antibody conjugates expressed as percentage ± std.dev.

Fig. 6 shows blood clearance of ^{111}In-1033-rituximab antibody conjugates expressed as % injected dose/gram ± std.dev.

Fig. 7 shows biodistribution of ^{111}In-1033-rituximab (4.6 1033/IgG) in rats.

Fig. 8 shows HPLC size exclusion separation of blood samples drawn from a rat injected with ^{111}In-1033-rituximab (4.6 1033/IgG).

Description of Preferred Embodiments

With the present invention it is possible to improve the tumour to non-tumour ratio of cytotoxic targeting agents in the treatment of disseminated haematological carcinomas, in particular lymphomas, by reducing the concentration of the cytotoxic medical agent in the blood circulation after administrations of a cytotoxic agent and thereby facilitating a higher dosage and hence a more effective treatment regime without exposing the vital organs to higher toxicity. Furthermore, the present invention presents new medicals and the use of these agents in the treatment of lymphatic cancer and NHL, in particular.

In one embodiment, the radiolabelled anti-lymphoma antibody is given in a single dose which is limited to what is regarded as tolerable to the patient without reconstitution of hematopoietic function, through bone marrow transplantation, or by some other means; "low dose". The dose range will be 10-20 MBq/ kg bodyweight of ^{90}Y-anti-lymphoma antibody, preferably 11-15 MBq/kg and the range for ^{111}In-anti-lymphoma antibody for targeting localisation will be 20 -250 MBq, preferable 50-150 MBq. In this embodiment, extracorporeal clearance of non-bound

radiolabelled therapeutic or diagnostic antibody is optional.

In another embodiment, the radiolabelled anti-lymphoma antibody is given in a single dose designated to deliver a high amount of radioactivity to the patient. This "high dose method" has to be combined with means of reconstituting the bone marrow or by reducing the radiation effect on bone marrow preferably by the use of the Mitradep® system. For ^{90}Y-anti-lymphoma antibodies, "high dose" means a single dose exceeding 20 MBq/ kg body weight.

In a preferred embodiment, ^{111}In-anti-lymphoma at a dose of 50-150 MBq is combined with a "high dose" (> 20 MBq/ kg body weight) of ^{90}Y-anti-lymphoma antibody, either given in sequence by a time interval of 6-8 days or given simultaneously.

The following embodiments of the invention also serve to explain the details of the invention.

Lymphomas are tumours originating from lymphocytes. The normal counterparts of lymphomas, i.e. the normal lymphocytes, arise from pluripotent stem cells in the bone marrow and differentiate to fully mature lymphocytes. During their differentiation they express different cell surface antigens (CD-antigens) some of which is lineage and/or stage specific. Lymphomas can arise from lymphocytes in various differentiation stages, and often presents the CD-antigens expressed at this stage. These CD-antigens cannot only be used for diagnostic purposes but also as targets for different kinds of antibody therapy.

The study of human leukocyte antigens, predominantly by monoclonal antibody techniques, is a rapidly changing area of basic research and clinical investigation. Leukocyte surface molecules defined by antibodies have been assigned cluster differentiation (CD) numbers (CD-antigens) at a series of international workshops (Paris, 1982; Boston, 1984; Oxford, 1986; Vienna, 1989, Osaka,

1996). The CD classification of these antigens has become the standard form in published literature and provides a basis for standardization of clinical reporting. The current CD classification is presented in the form of a list, with a brief summary of each antigen beside each entry.

CD molecule	Alternate Names	Locus ID	Past Guides
CD1a	R4; HTA1	909	CD1a
CD1b	R1	910	CD1b
CD1c	M241; R7	911	CD1c
CD1d	R3	912	CD1d
CD1e	R2	913	CD1e
CD2	CD2R; E-rosette receptor; T11; LFA-2	914	CD2
CD3delta	CD3d	915	
CD3epsilon	CD3e	916	
CD3gamma	CD3g	917	
CD4	L3T4; W3/25	920	CD4
CD5	Leu-1; Ly-1; T1; Tp67	921	CD5
CD6	T12	923	CD6
CD7	gp40	924	
CD8alpha	Leu2; Lyt2; T cell co-receptor; T8	925	
CD8beta	Leu2; CD8; Lyt3	926	
CD9	DRAP-27; MRP-1; p24	928	CD9
CD10	EC 3.4.24.11; neprilysin; CALLA; enkephalinase; gp100; NEP	4311	
CD11a	AlphaL integrin chain; LFA-1alpha	3683	CD11a
CD11b	AlphaM integrin chain; AlphaM-beta2; C3biR; CR3; Mac-1; Mo1	3684	CD11b
CD11c	AlphaX integrin chain; Axb2; CR4; leukocyte surface antigen p150,95	3687	CD11c

CD molecule	Alternate Names	Locus ID	Past Guides
CDw12	p90-120	23444	CDw12
CD13	APN; EC 3.4.11.2; gp150	290	CD13
CD14	LPS-R	929	CD14
CD15u	Sulphated CD15		
CD16a	FCRIIIA	2214	
CD16b	FCRIIIB	2215	
CDw17	LacCer		CDw17
CD18	CD11a beta subunit; CD11b beta subunit; CD11c beta subunit; beta-2 integrin chain	3689	CD18
CD19	B4	930	CD19
CD20	B1; Bp35	931	
CD21	C3d receptor; CR2; EBV-R	1380	CD21
CD22	BL-CAM; Lyb8	933	CD22
CD23	B6; BLAST-2; FceRII; Leu-20; Low affinity IgE receptor	2208	CD23
CD24	BA-1; HSA	934	CD24
CD25	IL-2R alpha chain; IL-2R; Tac antigen	3559	CD25
CD26	EC 3.4.14.5; ADA-binding protein; DPP IV ectoenzyme	1803	CD26
CD27	S152; T14	939	CD27
CD28	T44; Tp44	940	CD28
CD29	Platelet GPIIa; VLA-beta chain; beta-1 integrin chain	3688	
CD30	Ber-H2 antigen; Ki-1 antigen	943	CD30
CD31	GPiia'; endocam; PECAM-1	5175	CD31
CD32	FCR II; Fc gamma RII	2212	
CD33	gp67; p67	945	CD33
CD34	gp105-120	947	CD34
CD35	C3bR; C4bR; CR1; Immune Adherence Receptor	1378	CD35

CD molecule	Alternate Names	Locus ID	Past Guides
CD36	GPIIIb; GPIV; OKM5-antigen; PASIV	948	CD36
CD37	gp52-40	951	CD37
CD38	T10; cyclic ADP-ribose hydrolase	952	CD38
CD39		953	
CD40	Bp50	958	CD40
CD41	GPIIb; alpha IIb integrin chain	3674	
CD42a	GPIX	2815	CD42a
CD42b	GPIbalpha; Glycocalicin	2811	CD42b
CD42c	GPIb-beta	2812	CD42c
CD42d	GPV	2814	CD42d
CD43	gpL115; leukocyte sialoglycoprotein; leukosialin; sialophorin	6693	CD43
CD44	ECMR III; H-CAM; HUTCH-1; Hermes; Lu, In-related; Pgp-1; gp85	960	CD44
CD44R	CD44v; CD44v9	960	CD44R
CD45	B220; CD45R; CD45RA; CD45RB; CD45RC; CD45RO; EC 3.1.3.4; LCA; T200; Ly5	5788	CD45
CD46	MCP	4179	CD46
CD47R	Rh-associated protein; gp42; IAP; neurophilin; OA3; MEM-133; formerly CDw149	961	CD47
CD48	BCM1; Blast-1; Hu Lym3; OX-45	962	CD48
CD49a	Alpha-1 integrin chain; VLA-1 alpha chain	3672	
CD49b	Alpha-2 integrin chain; GPIa; VLA-2 alpha chain	3673	
CD49c	Alpha-3 integrin chain; VLA-3 alpha chain	3675	
CD49d	Alpha-4 integrin chain; VLA-4 alpha chain	3676	CD49d
CD49e	Alpha-5 integrin chain; FNR alpha	3678	

CD molecule	Alternate Names	Locus ID	Past Guides
	chain; VLA-5 alpha chain		
CD49f	Alpha-6 integrin chain; Platelet gpI; VLA-6 alpha chain	3655	
CD50	ICAM-3	3385	CD50
CD51	VNR-alpha chain; alpha V integrin chain; vitronectin receptor	3685	
CD52		1043	CD52
CD53		963	CD53
CD54	ICAM-1	3383	CD54
CD55	DAF	1604	CD55
CD56	Leu-19; NKH1; NCAM	4684	CD56
CD57	HNK1; Leu-7	964	
CD58	LFA-3	965	CD58
CD59	1F-5Ag; H19; HRF20; MACIF; MIRL; P-18; Protectin	966	CD59
CD60a	GD3		CDw60
CD60b	9-O-acetyl-GD3		CDw60
CD60c	7-O-acetyl-GD3		CDw60
CD61	CD61A; GPIIb/IIIa; beta 3 integrin chain	3690	
CD62E	E-selectin; ELAM-1; LECAM-2	6401	CD62E
CD62L	L-selectin; LAM-1; LECAM-1; Leu-8; MEL-14; TQ-1	6402	CD62L
CD62P	P-selectin; GMP-140; PADGEM	6403	CD62P
CD63	LIMP; MLA1; PTLGP40; gp55; granulophysin; LAMP-3; ME491; NGA	967	CD63
CD64	FC gammaRI; FCR I	2209	CD64
CD65	Ceramide-dodecasaccharide; VIM-2		
CD65s	Sialylated-CD65; VIM2		
CD66a	NCA-160; BGP	634	CD66a
CD66b	CD67; CGM6; NCA-95	1088	CD66b

CD molecule	Alternate Names	Locus ID	Past Guides
CD66c	NCA; NCA-50/90	4680	CD66c
CD66d	CGM1	1084	CD66d
CD66e	CEA	1048	CD66e
CD66f	Pregnancy specific b1 glycoprotein; SP-1; PSG	5669	CD66f
CD68	gp110; macrosialin	968	CD68
CD69	AIM; EA 1; MLR3; gp34/28; VEA	969	CD69
CD70	CD27-ligand; Ki-24 antigen	970	
CD71	T9; transferrin receptor	7037	CD71
CD72	Ly-19.2; Ly-32.2; Lyb-2	971	
CD73	Ecto-5'-nucleotidase	4907	CD73
CD74	Class II-specific chaperone; Ii; Invariant chain	972	CD74
CD75	Lactosamines		
CD75s	Alpha-2,6-sialylated lactosamines (formerly CDw75 and CDw76)		CDw75; CDw76
CD77	Pk blood group antigen; BLA; CTH; Gb3		CD77
CD79a	Ig alpha; MB1	973	
CD79b	B29; Ig beta	974	
CD80	B7; BB1	941	CD80
CD81	TAPA-1	975	CD81
CD82	4F9;C33;IA4;KAI1; R2	3732	CD82
CD83	HB15	9308	CD83
CD84		8832	CD84
CD85	ILT/LIR family {Young NT/Parham P.2001.IMMUN} {Allan DS/Braud VM.2000.IMMUN}	10859	CD85
CD86	B7-2;B70	942	CD86
CD87	uPAR	5329	CD87
CD88	C5aR	728	CD88
CD89	Fcalpha-R; IgA Fc receptor;IgA	2204	CD89

CD molecule	Alternate Names	Locus ID	Past Guides
	receptor		
CD90	Thy-1	7070	CD90
CD91	ALPHA2M-R; LRP	4035	
CD92	CTL1; formerly CDw92	23446	CD92
CDw93		23447	CDw93
CD94	Kp43	3824	CD94
CD95	APO-1; Fas; TNFRSF6; APT1	355	CD95
CD96	TACTILE	10225	
CD97		976	CD97
CD98	4F2; FRP-1; RL-388	4198	CD98
CD99	CD99R; E2; MIC2 gene product	4267	
CD100	SEMA4D	10507	CD100
CD101	IGSF2; P126; V7	9398	CD101
CD102	ICAM-2	3384	CD102
CD103	ITGAE; HML-1; integrin alphaE chain	3682	CD103
CD104	beta 4 integrin chain; TSP-1180; beta 4	3691	
CD105	endoglin	2022	CD105
CD106	INCAM-110; VCAM-1	7412	
CD107a	LAMP-1	3916	CD107a
CD107b	LAMP-2	3920	CD107b
CD108	SEMA7A; JMH human blood group antigen; formerly CDw108	8482	CD108
CD109	8A3; E123; 7D1		
CD110	MPL; TPO-R; C-MPL	4352	
CD111	PVRL1; PRR1; HevC; nectin-1; HIgR	5818	
CD112	HVEB; PRR2; PVRL2; nectin 2	5819	
CD113	Reserved		
CD114	CSF3R; HG-CSFR; G-CSFR	1441	CD114
CD115	c-fms; CSF-1R; M-CSFR	1436	

CD molecule	Alternate Names	Locus ID	Past Guides
CD116	GM-CSF receptor alpha chain	1438	CD116
CD117	c-KIT; SCFR	3815	CD117
CD118	Reserved		
CDw119	IFNgR; IFNgRa	3459	
CD120a	TNFRI; p55	7132	
CD120b	TNFRII; p75; TNFR p80	7133	
CD121a	IL-1R; type 1 IL-1R	3554	
CDw121b	IL-1R, type 2	7850	
CD122	IL-2Rbeta	3560	CD122
CD123	IL-3Ralpha	3563	
CD124	IL-4R	3566	CD124
CDw125	IL-5Ralpha	3568	CDw125
CD126	IL-6R	3570	CD126
CD127	IL-7R; IL-7R alpha; p90 I17 R	3575	CD127
CDw128a	CXCR1; IL-8RA	3577	
CDw128b	CXCR2; IL-8RB	3579	
CD129	Reserved		
CD130	gp130	3572	CD130
CD131	common beta subunit	1439	CDw131
CD132	IL2RG; common cytokine receptor gamma chain; common gamma chain	3561	CD132
CD133	PROML1; AC133; hematopoietic stem cell antigen; prominin-like 1	8842	
CD134	OX40	7293	
CD135	flt3; Flk-2; STK-1	2322	CD135
CDw136	msp receptor; ron; p158-ron	4486	CDw136
CDw137	4-1BB; ILA	3604	CDw137
CD138	heparan sulfate proteoglycan; syndecan-1	6382	
CD139		23448	CD139

CD molecule	Alternate Names	Locus ID	Past Guides
CD140a	PDGF-R; PDGFRa	5156	
CD140b	PDGFRb	5159	
CD141	fetomodulin; TM	7056	CD141
CD142	F3; coagulation Factor III; thromboplastin; TF	2152	CD142
CD143	EC 3.4.15.1; ACE; kininase II; peptidyl dipeptidase A	1636	CD143
CD144	cadherin-5; VE-Cadherin	1003	CD144
CDw145			
CD146	MCAM; A32; MUC18; Mel-CAM; S-endo	4162	CD146
CD147	5A11; Basigin; CE9; HT7; M6; Neurothelin; OX-47; EMMPRIN; gp42	682	CD147
CD148	HPTP-eta; DEP-1; p260	5795	CD148
CDw149	new designation is CD47R		
CD150	SLAM; IPO-3; fomerly CDw150	6504	CDw150
CD151	PETA-3; SFA-1	977	CD151
CD152	CTLA-4	1493	CD152
CD153	CD30L	944	
CD154	CD40L; T-BAM; TRAP; gp39	959	
CD155	PVR	5817	
CD156a	ADAM8; MS2 human; fomerly CD156	101	CD156a
CD156b	ADAM17; TACE; cSVP	6868	
CD157	BP-3/IF-7; BST-1; Mo5	683	CD157
CD158	KIR family (detailed nomenclature to be published)		KIR Family
CD159a	NKG2A	3821	
CD160	BY55 antigen; NK1; NK28	11126	
CD161	KLRB1; NKR-P1A; killer cell lectin-like receptor subfamily B, member 1	3820	CD161
CD162	PSGL-1, PSGL	6404	CD162
CD162R	PEN5 (a post-translational	6404	

CD molecule	Alternate Names	Locus ID	Past Guides
	modification of PSGL-1)		
CD163	GHI/61; M130; RM3/1	9332	
CD164	MUC-24; MGC-24v	8763	
CD165	AD2; gp37	23449	CD165
CD166	BEN; DM-GRASP; KG-CAM; Neurolin; SC-1; ALCAM	214	CD166
CD167a	trkE; trk6; cak; eddr1; DDR1; MCK10; RTK6; NTRK4	780	
CD168	HMMR; IHABP; RHAMM	3161	
CD169	sialoadhesin; siglec-1	6614	
CD170	Siglec-5	8778	
CD171	L1; L1CAM; N-CAM L1	3897	
CD172a	SIRP alpha	8194	
CD173	Blood group H type 2		
CD174	Lewis y	2525	
CD175	Tn		
CD175s	Sialyl-Tn		
CD176	TF		
CD177	NB1		
CD178	fas-L; TNFSF6; APT1LG1; CD95-L	356	
CD179a	VpreB; VPREB1; IGVPB	7441	
CD179b	IGLL1; lambda5; immunoglobulin omega polypeptide; IGVPB; 14.1 chain	3543	
CD180	LY64; RP105	4064	
CD183	CXCR3; GPR9; CKR-L2; IP10-R; Mig-R	2833	
CD184	CXCR4; fusin; LESTR; NPY3R; HM89; FB22	7852	
CD195	CCR5	1234	
CDw197	CCR7	1236	
CD200	OX2	4345	

CD molecule	Alternate Names	Locus ID	Past Guides
CD201	EPC R	10544	
CD202b	tie2; tek	7010	
CD203c	NPP3; PDNP3; PD-Ibeta; B10; gp130RB13-6; ENPP3; bovine intestinal phosphodiesterase	5169	
CD204	macrophage scavenger R	4481	
CD205	DEC205	4065	
CD206	MRC1; MMR	4360	
CD207	Langerin	50489	
CD208	DC-LAMP	27074	
CD209	DC-SIGN	30385	
CDw210	IL-10 R	3587; 3588	
CD212	IL-12 R	3594	
CD213a1	IL-13 R alpha 1	3597	
CD213a2	IL-13 R alpha 2	3598	
CDw217	IL-17 R	23765	
CD220	Insulin R	3643	
CD221	IGF1 R	3480	
CD222	Mannose-6-phosphate/IGF2 R	3482	
CD223	LAG-3	3902	
CD224	GGT; EC2.3.2.2	2678	
CD225	Leu13	8519	
CD226	DNAM-1; PTA1; TLiSA1	10666	
CD227	MUC1; episialin; PUM; PEM; EMA; DF3 antigen; H23 antigen	4582	
CD228	melanotransferrin	4241	
CD229	Ly9	4063	
CD230	Prion protein	5621	
CD231	TM4SF2; A15; TALLA-1; MXS1; CCG-B7; TALLA	7102	

CD molecule	Alternate Names	Locus ID	Past Guides
CD232	VESP R	10154	
CD233	band 3; erythrocyte membrane protein band 3;AE1; SLC4A1; Diego blood group; EPB3	6521	
CD234	Fy-glycoprotein; Duffy antigen	2532	
CD235a	Glycophorin A	2993	
CD235b	Glycophorin B	2994	
CD235ab	Glycophorin A/B crossreactive mabs		
CD236	Glycophorin C/D		
CD236R	Glycophorin C	2995	
CD238	Kell	3792	
CD239	B-CAM	4059	
CD240CE	Rh30CE	6006	
CD240D	Rh30D	6007	
CD240DCE	Rh30D/CE crossreactive mabs		
CD241	RhAg	6005	
CD242	ICAM-4	3386	
CD243	MDR-1	5243	
CD244	2B4; NAIL; p38	51744	
CD245	p220/240		
CD246	Anaplastic lymphoma kinase	238	
CD247	Zeta chain	919	

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The expression "the group of CD1 to CD247" as used herein means all the CD molecules on the list above.

In the most preferred embodiment the anti-lymphoma antibody is directed against CD19, CD20, CD22, CD 30, in particular CD 20.

In the present patent application an immunotargeting agent (immunoconjugate) is an agent carrying a cytotoxic

moiety that, contrary to common cytotoxic medical agents, binds specifically to lymphatic tumor cell with a high affinity and which could be administered intravenously to a human being. In a preferred application the immunotargeting agents are antibodies, which could be of different isotypes and could originate from any species. Of particular interest are the monoclonal antibodies and derivatives thereof. The latter include fragments such as the $F(ab`)_2$, F(ab`), F(ab) and the like. They also include genetically engineered hybrids or chemically synthesized peptides based on the specificity of the antigen binding region of one or several target specific monoclonal antibodies, e.g. chimeric or humanized antibodies, single chain antibodies etc.

The biomolecule binding moiety, which is an anti-lymphoma antibody reactive moiety, is bound or conjugated to the anti-lymphoma antibody, either covalently or non-covalently with an affinity binding constant of at least 10^9M^{-1}.

The term "anti-lymphoma antibody" used herein is intended to mean an antibody with the ability of specific binding to a CD antigen on lymphoma tumour cells with an affinity binding constant of at least $5x10^6M^{-1}$.

The term "variants" of the anti-lymphoma antibody as used herein means any modifications, fragments or derivatives thereof having the same or esentially similar affinity binding constant when binding to the CD antigen molecule, i.e. an affinity binding constant of at least $5x10^6M^{-1}$.

Any of these variants could have been modified by the coupling of various number of polyethylene glycol chains in order to optimise the half-life in body fluid and the retention of the antibody or antibody fragments or derivatives, in the tumor tissue. In the most preferred application the antibodies or antibody derivatives should allow for the attachment of a sufficient number of biotin residues to be used for extracorporeal removal

through interaction with immobilized avidin, without significantly diminishing the binding properties of the targeting agent.

In order to enhance the specificity, tumour specific monoclonal antibodies are used as a carrier (immunoconjugates) of various cytotoxic moieties, such as, but not limited to, radio nuclides, chemotherapy drugs, synthetic or natural occurring toxins, immunosuppressive agents, immunostimulating agents and enzymes used in pro-drug protocols. The cytotoxic moiety is preferable a radionuclide such as a gamma-emitter e.g. iodine-131 or metal ion conjugate, where the metal is selected from a beta-particle emitter, such as yttrium or rhenium. US. Patent No. 4,472,509, Gansow, et al., discloses the use of diethylenetriaminepentaacetic acid (DTPA) chelating agents for the binding of radio metals to monoclonal antibodies. The patent is particularly directed to a purification technique for the removal of non-bonded and adventitiously bonded (non-chelated) metal from radiopharmaceuticals but is illustrative of art recognized protocols for preparation of radionuclide labelled antibodies.

According to such general procedures, an antibody specifically reactive with the target tissue associated antigen is reacted with a quantity of a selected bifunctional chelating agent having protein binding and metal binding functionalities to produce a chelator/antibody conjugate. In conjugating the antibodies with the chelators an excess of chelating agent is reacted with the antibodies, the specific ratio being dependent upon the nature of the reagents and the desired number of chelating agents per antibody. It is a requirement that the radionuclides are bound by chelation (for metals) or covalent bonds in such a manner that they do not become separated from the biotinylation/radiolabeling compound under the conditions that the biomolecule conjugates is used (e.g. in patients). Thus, the most stable chelates or covalent bonding arrangements are preferred. Examples

of such binding/bonding moieties are: aryl halides and vinyl halides for radionuclides of halogens; N_2S_2 and N_3S chelates for Tc and Re radionuclides; amino-carboxy derivatives such as EDTA , DTPA, derivatives Me-DTPA and Cyclohexyl-DTPA, and cyclic amines such as NOTA, DOTA, TETA, CITC-DTPA, and triethylenetetraaminehexaacetic acid derivatives (Yuangfang and Chuanchu, Pure & Appl. Chem. 63, 427-463, 1991) for In, Y, Pb, Bi, Cu, Sm, and Lu radionuclides.

Beta radiation emitters, which are useful as cytotoxic agents, include isotopes such as scandium-46, scandium-47, scandium-48, copper-67, gallium-72, gallium-73, yttrium-90, ruthenium-97, palladium-100, rhodium-101, palladium-109, samarium-153, rhenium-186, rhenium-188, rhenium-189, gold-198, radium-212 and 212-lead. The most useful gamma emitters are iodine-131 and indium-m114. Other metal ions useful with the invention include alpha radiation emitting materials such as 212-bismuth, 213-bismuth, and At-211 as well as positron emitters such as gallium-68 and zirconium-89.

In another embodiment of the invention, radionuclide-labelled targeting agents are useful not only in the treatment of lymphatic cancers, but also for imaging of such cancers.

At a suitable time after administration, " cytotoxic targeting agents" will be cleared from the blood system by extracorporeal means. To facilitate the extracorporeal depletion an apparatus for extracorporeal circulation of whole blood or plasma will be connected to the patient through tubing lines and blood access device(s). Such an apparatus should provide conduits for transporting the blood to an adsorption device and conduits for returning the processed blood or plasma to the patient. In the case plasma is processed through the adsorption device, a plasma separation device is needed as well as means of mixing the concentrated blood with processed plasma. The

later normally is achieved by leading the two components into an air-trap where the mixing occurs.

In the case where whole blood is processed an ordinary dialysis machine can constitute the base for such an apparatus. Dialysis machines are normally equipped with all the necessary safe guards and monitoring devices to meet patient safety requirements as well as easy handling of the system. Hence, in a preferred embodiment whole blood is processed and a standard dialysis machine is utilised with only minor modifications of the hardware. However, such a machine requires a new program fitted to the new intended purpose.

In addition to the apparatus, special blood line tubings suitable for the intended flow and distance from the patient and the machine is needed. These line tubings could be made of any material compatible with blood or plasma and would include material used in ordinary tubings used in dialysis.

Blood access could be achieved through peripheral vein catheters or if higher blood flow is needed through central vein catheters such as, but not limited to, subclavian or femoral catheters.

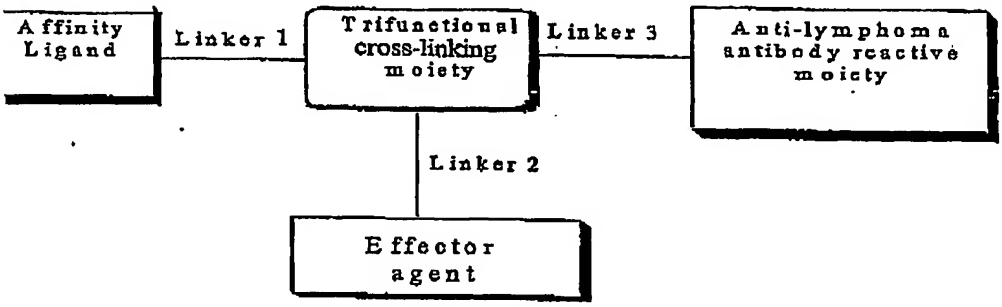
For affinity adsorbents, the matrix may be of various shape and chemical composition. It may for example constitute a column house filled with particulate polymers, the latter of natural origin or artificially made. The particles may be macroporous or their surface may be grafted, the latter in order to enlarge the surface area. The particles may be spherical or granulated and be based on polysaccharides, ceramic material, glass, silica, plastic, or any combination of these or alike material. A combination of these could for example be solid particles coated with a suitable polymer of natural origin or artificially made. Artificial membranes may also be used. These may be flat sheet membranes made of cellulose, polyamide, polysulfone, polypropylene or other types of material which are sufficiently inert, biocompatible, non-

toxic and to which the receptor could be immobilized either directly or after chemical modification of the membrane surface. Capillary membranes like the hollow fibers made from cellulose, polypropylene or other materials suitable for this type of membranes may also be used. A preferred embodiment is a particulate material based on agarose and suitable for extracorporeal applications.

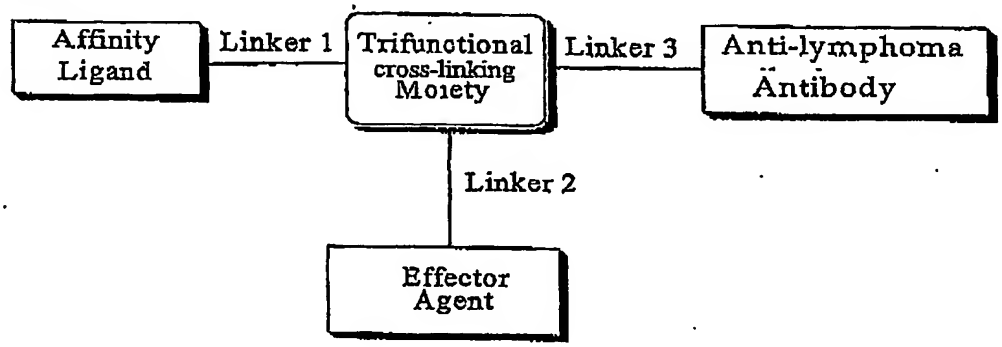
In one embodiment an affinity label is attached to the anti-lymphoma antibody and the adsorption device contains an immobilized receptor binding specifically to the affinity ligand. Any type of affinity ligand/immobilized receptor combinations such as "antibodies and antigens/haptens" and "protein and co-factors" could be used in the this application, provided that the they exhibit a sufficiently high binding affinity and selectively to the tumor markers and that the affinity ligand-receptor interaction is not interfered with by blood or other body fluids or tissues being in contact with the immunotargeting agent and/or the device.

In one of the most preferred applications, the affinity ligand/immobilized receptor combination is biotin or biotin derivatives and biotin binding molecules and in particular where the affinity ligand is biotin or derivatives thereof and the immobilized receptor is avidin or streptavidin or any other biotin binding molecule. The affinity ligand pairs of biotin/avidin and biotin/streptavidin are often used with biomolecules. The very strong interaction (i.e. $K = 10^{13} - 10^{15}$ M^{-1}) of biotin with the proteins avidin and streptavidin (Green, Methods Enzymol. 184, 51-67, 1990; Green, Adv. Prot. Chem. 29, 85-133, 1975) provides a foundation for their use in a large number of applications, both for *in vitro* and *in vivo* uses. A further application of the invention is the simultaneous removal of several different biotinylated "anti-cancer agents" through the same extracorporeal procedure.

The reagent according to the present invention is schematically shown below, wherein the biomolecule reactive moiety is an anti-lymphoma reactive moiety.



The medical agent according to the present invention is schematically shown below, wherein an anti-lymphoma antibody is bound or conjugated to the reagent via the anti-lymphoma antibody reactive moiety of the reagent.



In the schematically shown reagent and medical agent, respectively, the different components are presented in more detail below.

The anti-lymphoma antibody reactive moiety is chosen from a group of active esters consisting of N-hydroxysuccinimide esters, sulfo-N-hydroxysuccinimide esters, and phenolic esters; aryl and alkyl imidates; alkyl or aryl isocyanates or isothiocyanates reacting with amino groups on the anti-lymphoma antibody, or maleimides or alpha-haloamides reacting with sulfhydryl groups on the

anti-lymphoma antibody; or aryl or alkylhydrazines or alkyl or arylhydroxylamines reacting with aldehyde or ketone groups naturally occurring or synthetically produced on the anti-lymphoma antibody, or variants thereof.

The effector agent is a radionuclide binding moiety, optionally provided with a radionuclide, a synthetic or naturally occurring toxin, an enzyme capable of converting pro-drugs to active drugs, immunosuppressive or immunostimulating agents, radiosensitizers, enhancers for X-ray or MRI or ultrasound, non-radioactive elements, which can be converted to radio active elements by means of external irradiation after that the anti-lymphoma antibody carrying said element has been accumulated to specific cells or tissues, or photoactive compounds or compounds used in photo imaging or photo dynamic therapy, or any other molecule having the same or similar effect, directly or indirectly, on lymphoma cells or lymphoma tissues. More precisely, the effector agent comprises Me-DTPA, CITC-DTPA, and cyclohexyl-DTPA.

The affinity ligand can be any moiety that binds with another molecule with an affinity constant of 10^6 M^{-1} or higher. A preferred affinity ligand is a moiety which binds specifically to avidin, streptavidin, or any other derivatives, mutants or fragments of avidin or streptavidin having essentially the same binding function to the affinity ligand. Preferably, the affinity ligands is biotin, or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin. Said biotin derivative may be chosen from the group consisting of a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin.

The anti-lymphoma antibody having ability to be conjugated to said anti-lymphoma antibody reactive moiety is interacting with one or more different cell surface antigen(s) present on the surface of lymphoma tumour

cells, said one or more cell surface antigen(s) being one or more different CD antigen(s), or variants thereof.

The trifunctional cross-linking moiety is chosen from the group consisting or triaminobenzene, tricarboxybenzene, dicarboxyanyline and diaminobenzoic acid.

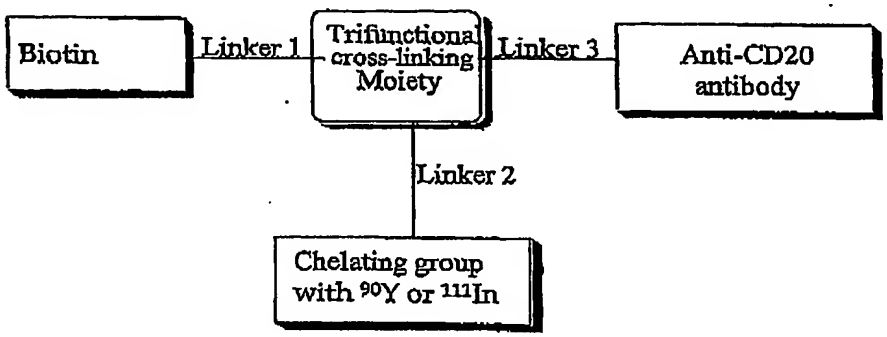
Linker 1 is a chemical moiety that is an attaching moiety and spacer between the trifunctional cross-linking moiety and the affinity ligand, preferably a biotin moiety, such that binding with avidin or streptavidin, or any other biotin binding species, is not diminished by steric hindrance. Linker 1 may also impart increased water solubility and biotinidase stabilization, preferably against cleavage by biotinidase by introduction of an alpha carboxylate or an N-methyl group. Further, it contains hydrogen bonding atoms, preferably ethers or tioethers, or ionisable groups, preferably carboxylate, sulfonates, or ammonium groups to aid in water solubilisation of the biotin moiety.

Linker 2, if present, is a chemical moiety that is used to attach the radionuclide binding moiety to the trifunctional cross-linking moiety. It provides a spacer length of 1-25 atoms, preferably a length of 6-18 atoms, or groups of atoms. Linker 2 may also impart increased water solubility due to the presence of hydrogen bonding atoms, preferably eters or bioeters, or ionisable groups, to aid in water solubilisation.

Linker 3 may not be required, but in cases where it is advantageous to have, it is a chemical moiety used to attach the biomolecule reactive moiety to the trifunctional cross-linking moiety. Linker 3 may be used as a spacer of a length of 1-25 atoms, preferably 6-18 atoms, or groups of atoms and/or it may be used to increase the water solubility of the compound due to the presence of hydrogen bonding atoms, such as eters or tioeters, or ionisable groups, preferably carboxylate, sulfonates, or ammonium groups to aid in water solubilisation.

Moreover, the reagent according to the present invention may contain more than one affinity ligand and/or more than one effector agent bound to a trifunctional or tetrafunctional cross-linking moiety.

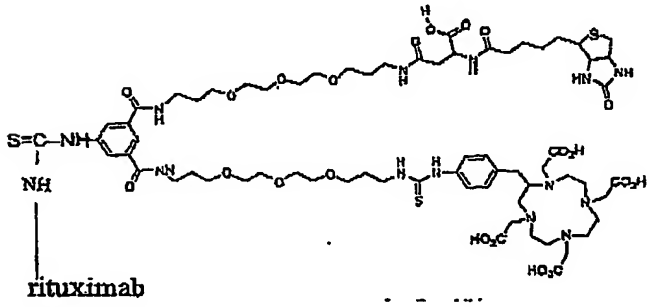
A preferred embodiment of the medical agent according to the present invention has the following schematic structure:



where the chelating group is, but not limited to, any of the following compounds: aryl halides and vinyl halides for radionuclides of halogens; N_2S_2 and N_3S chelates for Tc and Re radionuclides; amino-carboxy derivatives such as EDTA, DTPA, derivatives Me-DTPA and Cyclohexyl-DTPA , and cyclic amines such as NOTA, DOTA, TETA, CITC-DTPA, and triethylenetetraaminehexaacetic acid derivatives (Yuangfang and Chuanchu, Pure & Appl. Chem. 63, 427-463, 1991) for In, Y, Pb, Bi, Cu, Sm, Lu radionuclides and where the radionuclide is, but not limited, any of the following elements: Beta radiation emitters, which are useful as cytotoxic agents, include isotopes such as scandium-46, scandium-47, scandium-48, copper-67, gallium-72, gallium-73, yttrium-90, ruthenium-97, palladium-100, rhodium-101, palladium-109, samarium-153, rhenium-186, rhenium-188, rhenium-189, gold-198, radium-212 and 212 lead. The most useful gamma emitters are iodine-131 and indium-m114. Other metal ions useful with the invention include alpha radiation emitting materials

such as 212-bismuth, 213-bismuth, and At-211 as well as positron emitters such as gallium-68 and zirconium-89.

In the most preferred embodiment of the present invention the medical agent is the rituximab conjugate with 1-5 groups of 3-(13'-ThioureabenzylDOTA)Trioxadiamine-1-(13''-Biotin-Asp-OH)Trioxadiamine-5-isothiocyanato-Aminoisophthalate (see below). The radionuclide is ^{90}Y for therapeutic application and ^{111}In for in vivo diagnostic application. In the very most preferred embodiment the rituximab conjugate contains 1.5 - 3.5 groups of 3-(13'-ThioureabenzylDOTA)Trioxadiamine-1-(13''-Biotin-Asp-OH)Trioxadiamine-5-isothiocyanato-Aminoisophthalate.



Ibritumomab is also effective as anti-lymphoma antibody in the medical agent.

Examples

The following examples shall not be construed as limiting the invention, but should be regarded as evidence for the applicability of the invention.

Example 1 - Conjugation and radiolabelling of rituximab.

In this and subsequent examples, Indium-111 has in some instances been used as a substitute for Yttrium-90, because the former is a gamma-emitter and possesses less radiation hazard than Yttrium-90.

The monoclonal antibody, Rituximab was conjugated with 3-(13'-ThioureabenzylDOTA)Trioxadiamine-1-(13"-Biotin-Asp-OH)trioxadiamine-5-Isothiocyanato-Aminoisophtalate (MitraTag-1033), for short also called "1033" in the following, using the method described by Wilbur D.S et al in Bioconjugate Chem. 13:1079-1092, 2002. A 5 mg quantity of the monoclonal antibody was dialysed against 1L metal free HEPES with a minimum of 5 buffer changes over 3 days at 4°C. A solution of MitraTag-1033 was made in water, and an appropriate volume was added to the antibody solution. After incubation overnight at room temperature, the antibody-conjugate was dialysed against 1L metal free 500 mM ammonium acetate buffer pH 5.3 with a minimum of 3 buffer changes over 3 days at 4°C. The demetalated conjugated antibody was stored at 4-8 °C until used in radiolabelling experiments.

275 µl antibody conjugate (13.75 µg; 1033-Rituximab) in 500 mM ammonium acetate buffer pH 5.3 was mixed with 15 µl $^{111}InCl_3$ (or $^{90}YCl_3$) in 50 mM HCl. The labelling was conducted at 45 °C for 16 minutes. 28 µl DTPA was added to stop reaction. The quality of the radio conjugate was determined by TLC and HPLC. The number of MitraTag-1033 per monoclonal antibody molecule was determined by the HABA method.

Example 2 - Binding of the 1033-conjugated monoclonal antibody to an avidin-adsorbent.

The fraction of the 1033-rituximab radio conjugate binding to the Avidin-adsorbent utilised in the Mitradep® device, was analysed utilising micro-columns.

The non-bound protein fraction of a 2.4 conjugates/-IgG 1033-rituximab was 9 %, and of a 4.6 conjugates/IgG 1033-rituximab 3 %. This is well in line with a Poisson distribution of the conjugates. Hence, the above Rituximab conjugates should contain fractions, which are not labelled with MitraTag-1033. Hence, the non-binding fraction comply with the expected fraction of non-conjugated Rituximab i.e. the non-radioactive fraction.

More than 99 % of the radioactivity in a radiolabelled 1033-conjugate sample was bound to the microcolumn with the Avidin-adsorbent.

Example 3 - Depletion of 1033-rituximab conjugates during in vitro simulated treatments.

The depletion kinetics of 1033-rituximab during a patient treatment was simulated *in vitro* utilising a recirculation method based in the principles described by Schindhelm K. (Artificial Organs 13:21-27 (1989)).

The 1033-rituximab was diluted in a solution with the same relative viscosity as human blood, and was re-circulated *in vitro* through a small-scale model of the Mitradep® device. 125 ml of a blood substitute, containing 10 mg of 1033-rituximab, were re-circulated at 6.25 ml/min (corresponds to 100 ml/min in Mitradep®). Three reservoir volumes were processed. The levels of 1033-rituximab in the reservoir were monitored.

When two preparations of 1033-rituximab with different number of MitraTag™-1033 moieties per Rituximab molecule were analysed, the result presented in figure 1 were obtained. As seen, the depletion of 1033-rituximab is not different from the theoretical depletion line, i.e. all 1033-rituximab present in the solution passing through the device is removed. Studies with biotinylated human IgG has shown that an efficient depletion is obtained at a biotin/IgG ratio down to 1.4 biotin/IgG (lowest ratio tested).

It was concluded that 1033-rituximab could be efficiently removed during an extracorporeal affinity adsorption procedure utilising the device Mitradep®.

Example 4 - Binding of the 1033-conjugate to the target antigen CD20.

After conjugation with MitraTag™-1033, the 1033-rituximab conjugates were analysed for binding to the target antigen CD20 to confirm that the conjugation process has not denaturated the antigen binding. The CD20 antigen is not available in purified and soluble form.

Therefore, during testing the CD20 expressing B-cell lymphoma cell lines, Raji and/or SB, was utilised as targets.

The specificity of the antigen binding was analysed by immunofluoroscence in a flowcytometry (FACS) method. Briefly, the Raji cells were incubated with biotinylated-Rituximab and 1033-rituximab conjugates. After incubation, the cells were washed and incubated with fluorescence-labelled Avidin. After washing the cells were analysed in the FACS instrument. As positive control, a biotinylated mouse monoclonal antibody against CD20 was used, as negative control PBS buffer was used. For control of binding to F_c-receptors on the cells biotinylated normal human IgG was used. The results are presented in graphs where the x-axis presents the amount of fluorescence per cell on a logarithmic scale, and the Y-axis the number of cells displaying the specified fluorescence.

As seen in figure 2, no none-specific binding of Avidin to the cells was detected. Neither was binding to F_c-receptors seen utilising biotinylated human IgG. There was no significant difference in binding between the control mouse antibody, biotinylated Rituximab, or the two MitraTag™-1033 conjugates tested.

The specificity was also determined by analysing the binding of the conjugates to a CD20-positive cell line (SB) and a CD20-negative cell line (HSB) established from the same individual in an ELISA. The cells were dried into the wells of an ELISA plate. After incubation with 1033-rituximab conjugates, the bound antibodies were detected with an enzyme-conjugated Streptavidin. Biotinylated Rituximab and biotinylated normal human IgG were used as positive and negative control, respectively. As seen in figure 3, non-specific binding to the control cells was insignificant.

It was concluded that Rituximab retain the binding specificity to the antigen CD20 after conjugation with the MitraTag™-1033 reagent.

<u>Example 5 - Analyses of the affinity of the binding to the CD20 antigen</u>

The influence of the conjugation process on the binding affinity (strength) of Rituximab to the target antigen CD20 was studied utilising a competitive inhibition assay.

Briefly, increasing amounts of non-radiolabelled Rituximab and 1033-rituximab conjugates were mixed with a constant amount of ^{125}I-labelled Rituximab labelled utilising the Bolton-Hunter reagent. The mixtures were added to fixed SB lymphoma cells in 96 plate wells. After incubation for 2 hours at room temperature, the wells were washed, and the radioactivity bound to the cells was measured in an automatic NaI(Tl) scintillation well counter.

For each concentration of cold Rituximab and 1033-rituximab conjugates, the per cent inhibition of cell binding radioactivity was calculated. The % inhibition was plotted against concentration (figure 4), and the concentration required for 50 % inhibition (IC_{50}) was calculated from the graph (table 1). The IC_{50} is a measure of the relative affinity (avidity) of the tested antibody; a decrease of affinity is seen as an increased IC_{50} concentration. To be a significant change in affinity it is often stated that the difference in IC_{50} should be at least 10-fold.

Table 1		
Sample	IC_{50} (nM)	IC_{50} (relative rituximab)
Rituximab	26	1.0
1.6 1033-rituximab(1)	106	4.1
2.4 1033-rituximab	100	3.8
3.4 1033-rituximab	350	13.5
4.6 1033-rituximab	440	16.9
Human IgG	No inhibition	--

(1) 1.6 1033-rituximab denotes 1033-rituximab conjugated with 1.6 MitraTag/ rituximab

A slight decrease in affinity was seen for the 1.6- and 2.4 1033-rituximab conjugates, whereas the decrease for the 3.4- and 4.6- 1033-rituximab conjugates were slightly above ten-fold relative to the IC50 concentration of Rituximab. The affinity for the 3.4- and 4.6-1033-rituximab conjugates is probably still high enough to obtain a proper tumour uptake in patients.

It has been shown in clinical studies that a tenfold difference in affinity does not result in any significant difference in tumour uptake (ref. 6). Therefore, it was concluded that conjugation of Rituximab with up to 3-4 conjugates per antibody would not diminish the binding properties of the antibody *in vivo*.

Example 6 - Pharmacokinetics of MitraTag-1033 conjugates of rituximab.

Rats of the Spraque Dawley strain were injected intravenously with approximately 50 µg of 1033-rituximab (4.6 1033 moieties per antibody) labelled with 3 - 4 MBq ^{111}Indium mixed with 1.2 mg/rat of a non-conjugated Rituximab. Whole body (WB) imaging was performed using a scintillation camera (General Electric 400T, GE,

Milwaukee, WI, USA) equipped with a medium-energy collimator. Images were stored and analysed with Nuclear MAC 2.7 software. From images, the total number of counts in the entire body were obtained. After radioactivity decay correction and background subtraction, the counts were used for the calculation of activity retention (%) in the body. See Figure 5.

To define pharmacokinetics of ^{111}In-1033-rituximab and compared it with ^{111}In-DOTA-hMn14, about 0,2 ml blood was obtained from the periorbital venous plexa on following occasions: 10 min, 1, 8, 24, 48 and 96 hours after injection. The radioactivity was measured in an automatic NaI(Tl) scintillation well counter and expressed in percent of injected activity per gram blood (%/g) corrected for ^{111}In decay (figure 6).

Example 7 - Biodistribution of conjugates to organs and tissues.

At dissections, performed after 1, 8, 24, 48, and 96 hours post injection, organs and tissues of interest were removed, weighed and measured for radioactivity content. The radioactivity was measured in an automatic NaI(Tl) scintillation well counter, and the counts were corrected for decay. The distribution of the injected activity is shown in figure 7, and table 2.

Table 2	Uptake of ^{111}In-1033-rituximab (% injected dose/g)				
Tissue	1 h	8 h	24 h	48 h	96 h
Muscle	0,06	0,06	0,12	0,13	0,11
Kidney	0,98	0,91	0,83	0,86	1,00
Liver	1,22	1,79	1,86	1,92	3,42
Spleen	1,02	0,99	1,04	1,30	1,31
Bowel	0,10	0,29	0,32	0,29	0,20
Lymph nodes	0,26	1,03	1,99	2,88	2,54
Lung	0,74	0,89	0,71	0,52	0,38
Bone marrow	0,79	0,62	0,57	0,65	0,52

Example 8 – *In vivo* stability of the radiolabelled MitraTag-1033 antibody conjugates.

The stability of the MitraTag™-1033 moiety *in vivo* was determined by analysing the percentage of radioactivity in blood binding to Avidin-microcolumns.

About 0,1 ml blood was obtained from the periorbital venous plexa on following occasions: 1, 8, 24, 48, and 96 hours after injection. 50 µl blood was applied to a microcolumn with Avidin-agarose (0.3 ml adsorbent). After incubation for 10 minutes, the unbound radioactivity was washed off the column. The radioactivity in the column and the collected washing fluid was measured in an automatic NaI(Tl) scintillation well counter and the bound fraction was expressed in percent of the total radioactivity applied to the column.

Time post injection (hours)	% Avidin-binding	Range (%)	Animals analysed
0	99.2	99.1 - 99.3	-
1	99.4	99.4 - 99.5	3
8	99.4	99.4 - 99.4	3
24	99.3	99.2 - 99.4	2
48	99.1	98.9 - 99.3	3
96	98.5	97.7 - 99.1	3

Sample 0 is on the conjugate to be injected.

During the period studied, no reduction of the binding to Avidin of the radioactivity present in blood could be detected.

Therefore, it was concluded that the linkage between biotin and the DOTA chelate is stable in blood circulation up to 96 hours post injection.

When plasma was separation on a HPLC size exclusion column, no significant change in size distribution could be seen when a 10 min sample was compared with a 47 hours sample (Figure 8).

<u>Example 9 - Treatment regime in B-cell lymphoma according to the most preferred embodiment of the invention.</u>

The treatment regime can be separated in the following events:

- All patients will receive a dose of 250 mg/m^2 rituximab one week prior to therapy (day −7) in order to eliminate the circulating B-cells, immediately followed by a diagnostic dose of 50-150 MBq (1.5-4 mCi) ^{111}In-1033-rituximab.
- On day 0 all patients will receive 250 mg/m2 rituximab immediately followed by a therapeutic dose of ^{90}Y-1033-rituximab (>10MBq/kg bodyweight). Patients may, optionally, be administered a dose of 150-250 MBq (4-7 mCi) ^{111}In-1033-rituximab, which will be used for imaging for dosimetry.
- On day 1 or 2 , patients are treated with Mitradep®, allowing 3 blood volumes to pass the Mitradep® device.

CLAIMS

1. A reagent for conjugation to an anti-lymphoma antibody, wherein the reagent is a single molecule with at least three functional parts,

a) wherein a trifunctional cross-linking moiety is coupled to

b) an affinity ligand via a linker 1,

c) to an effector agent via a covalent bond, optionally via a linker 2,

e) and to a biomolecule reactive moiety, optionally via a linker 3, characterised in that, said biomolecule reactive moiety is an anti-lymphoma antibody reactive moiety being capable of forming a bond with an anti-lymphoma antibody, thereby forming a conjugate.

2. The reagent according to claim 1, wherein the anti-lymphoma antibody reactive moiety is chosen from a group of active esters consisting of N-hydroxysuccinimide esters, sulfo-N-hydroxysuccinimide esters, and phenolic esters; aryl and alkyl imidates; alkyl or aryl isocyanates or isothiocyanates reacting with amino groups on the anti-lymphoma antibody, or maleimides or alpha-haloamides reacting with sulfhydryl groups on the anti-lymphoma antibody; or aryl or alkylhydrazines or alkyl or arylhydroxylamines reacting with aldehyde or ketone groups naturally occurring or synthetically produced on the anti-lymphoma antibody.

3. The reagent according to claim 2, wherein said anti-lymphoma antibody reactive moiety also includes variants having essentially the same ability to bind to said anti-lymphoma antibody

4. The reagent according to any one of the preceding claims, wherein the anti-lymphoma antibody having ability to be conjugated to said anti-lymphoma antibody reactive moiety is interacting with one or more different cell surface antigen(s) present on the surface of lymphoma

tumour cells, said one or more cell surface antigen(s) being one or more different CD antigen(s).

5. The reagent according to claim 4, wherein said one or more CD antigen(s) is/are chosen from the group of CD1 to CD247, preferably CD19, CD20, CD22 and CD30, most preferably CD20.

6. The reagent according to any one of the preceding claims, wherein said anti-lymphoma antibody having ability to be conjugated to said anti-lymphoma antibody reactive moiety is ibritumomab or rituximab, preferably rituximab.

7. The reagent according to any one of the preceding claims, wherein said anti-lymphoma antibody also include variants thereof having the same or essentially the same ability to bind to both the anti-lymphoma antibody reacting moiety and to said cell surface antigen on the surface of lymphoma tumour cells.

8. The reagent according to any one of the preceding claims, wherein the bond formed between the anti-lymphoma antibody reactive moiety and the anti-lymphoma antibody either is covalent or non-covalent with a binding affinity constant of at least 10^8M^{-1}.

9. The reagent according to any one of claims 1-7, wherein the anti-lymphoma antibody or variants thereof binds to said cell surface antigens on lymphoma tumour cells with an affinity binding constant of at least $5x10^6M^{-1}$.

10. The reagent according to any one of the preceding claims, wherein the effector agent is a radionuclide binding moiety, optionally provided with a radionuclide, a synthetic or naturally occurring toxin, an enzyme capable of converting pro-drugs to active drugs, immunosuppressive or immunostimulating agents, radiosensitizers, enhancers for X-ray or MRI or ultrasound, non-radioactive elements, which can be converted to radio active elements by means of external irradiation after that the anti-lymphoma antibody carrying said element has been

accumulated to specific cells or tissues, or photoactive compounds or compounds used in photo imaging or photo dynamic therapy, or any other molecule having the same or similar effect, directly or indirectly, on lymphoma cells or lymphoma tissues.

11. The reagent according to claim 10, wherein the effector agent comprises aryl halides and vinyl halides for radio nuclides of halogens, N_2S_2 and N_3S chelatés for Tc and Re radionuclides, amino-carboxy derivatives, preferably EDTA and DTPA or derivatives thereof, and cyclic amines} preferably NOTA, DOTA and TETA, and derivatives thereof, for In, Y, Pb, Bi, Cu, Sm and Lu radionuclides, or any other radionuclide capable of forming a complex with said chelates.

12. The reagent according to claim 11, wherein the DTPA derivatives are Me-DTPA, CITC-DTPA, and cyclohexyl-DTPA.

13.The reagent according to any one of claims 10-12, wherein the effector agent comprises DOTA and is provided with Y-90 for therapeutic application or In-111 for diagnostic application.

14.The reagent according to any one of claims 10-13, wherein the effector agent is provided with positron imaging radionuclides, preferably F-18, Br-75, Br-76, and I-124; therapeutic radionuclides, preferably Y-90, I-131, In-114m, Re-186, Re-188, Cu-67, Sm-157, Lu-177, Bi-212, Bi-213, At-211, Ra-223; and gamma imaging radionuclides, preferably Tc-99m, In-111, I-123, and I-125.

15. The reagent according to any one of the preceding claims, wherein the affinity ligand is capable of binding with another molecule having affinity for said ligand with an affinity binding constant of at least $10^6 M^{-1}$

16. The reagent according to claim 15, wherein the affinity ligand is a moiety which binds specifically to avidin, streptavidin or any other derivatives, mutants or

fragments of avidin or streptavidin having essentially the same binding function to this affinity ligand.

17. The reagent according to claim 16, wherein the affinity ligand is biotin, or a biotin derivative having essentially the same binding function to avidin or streptavidin as biotin.

18. The reagent according to claim 17, wherein the biotin derivative is chosen from the group consisting of norbiotin, homobiotin, oxybiotin, iminobiotin, destibiotin, diaminobiotin, biotin sulfoxide, and biotin sulfone, or derivatives thereof having essentially the same binding function.

19. The reagent according to any one of the preceding claims, wherein the stability towards enzymatic cleavage, preferably against cleavage by biotinidase, of the bioatinamide bond to release biotin has been improved by using biotin derivatives, preferably norbiotin or homobiotin.

20. The reagent according to any one of the preceding claims, wherein the trifunctional cross-linking moiety is chosen from the group consisting of triaminobenzene, tricarboxybenzene, dicarboxyanyline and diaminobenzoic acid.

21. The reagent according to any one of the preceding claims, wherein linker 1 serves as an attaching moiety and a spacer between the trifunctional cross-linking moiety and the affinity ligand, preferably a biotin moiety, such that binding with avidin or streptavidin, or any other biotin binding species, is not diminished by steric hindrance.

22. The reagent according to any one of the preceding claims, wherein linker 1 contains hydrogen bonding atoms, preferably eters or tioeters, or ionisable groups preferably carboxylate, sulfonates, or ammonium groups to aid in water solubilisation of the biotin moiety.

23. The reagent according to claim 22, wherein stability towards enzymatic cleavage, preferably against cleavage by biotinidase, of the biotin amide bond to release biotin has been provided by introducing an alpha carboxylate or an N-metyl group in linker 1.

24. Linker according to any one of the preceding claims, wherein linker 2 provides a spacer length of 1-25 atoms, preferably a length of 6-18 atoms, or groups of atoms.

25. The reagent according to claim 24, wherein linker 2 contains hydrogen bonding atoms, preferably eters or tioeters, or ionisable groups, to aid in water solubilisation.

26. The reagent according to any one of claims 1-23, wherein linker 2 is excluded.

27. The reagent according to any one of the preceding claims, wherein linker 3 provides a spacer of a length of 1-25 atoms, preferably a length of 6-18 atoms, or groups of atoms.

28. The reagent according to claim 27, wherein linker 3 contains hydrogen bonding atoms such as eters or tioeters, or ionisable groups, preferably carboxylate, sulfonates, or ammonium groups to aid in water solubilisation.

29. The reagent according to any one of claims 1-26, wherein linker 3 is excluded.

30. The reagent according to any one of the preceding claims, wherein more than one affinity ligand and/or more than one effector agent are bound to a trifunctional or tetrafunctional cross-linking moiety.

31. The reagent according to any one of the preceding claims, wherein it is 3-(13'-thioureabenzyl(DOTA)trioxadiamine-1-(13"-Biotin-Asp-OH)trioxamine-5-Isothiocyanato-Aminoisophthalate, X or Y, preferably 3-(13'-thioureabenzyl(DOTA)trioxadiamine-1-(13"-Biotin-Asp-OH)trioxamine-5-Isothio-cyanato-Aminoisophthalate.

32. A medical agent comprising a reagent according to any one of claims 1-31 conjugated to said anti-lymphoma antibody via the anti-lymphoma antibody reactive moiety of said reagent.

33. The medical agent according to claim 32, wherein the anti-lymphoma antibody is interacting with one or more different cell surface antigen(s) present on the surface of lymphoma tumour cells, said one or more cell surface antigen(s) being one or more different CD antigen(s).

34. The medical agent according to claim 30, wherein said one or more CD antigen(s) is/are chosen from the group of CD1 to CD247, preferably CD19, CD20, CD22, and CD 30, most preferably CD20.

35. The medical agent according to any one of claims 32-34, wherein the anti-lymphoma antibody is ibritumomab or rituximab, preferably rituximab.

36. The medical agent according to any one of claims 32-35, wherein the anti-lymphoma antibody also includes variants thereof having the same or essentially the same ability to bind to both the anti-lymphoma antibody reacting moiety and said cell surface antigen on the surface of lymphoma tumour cells.

37. The medical agent according to any one of claims 32-36, wherein the bond formed between the anti-lymphoma antibody reactive moiety and the anti-lymphoma antibody either is covalent or non-covalent with a binding affinity constant of at least $10^8 M^{-1}$.

38. The medical agent according to any one of claims 32-36, wherein the anti-lymphoma antibody or variants thereof binds to said cell surface antigens on lymphoma tumour cells with an affinity binding constant of at least $5x10^6 M^{-1}$.

39. The medical agent according to any one of claims 29-33, wherein it is 3-(13'-thioureabenzyl(DOTA)trioxadiamine-1-(13''-Biotin-Asp-OH)trioxamine-5-Isothiocyanato-Aminoisophthalate-ibritomumab or 3-(13'-thiourea-

benzyl(DOTA)trioxadiamine-1-(13"-Biotin-Asp-OH)trioxamine-5-Isothio-cyanato-Aminoisophthalate-rituximab, preferably 3-(13'-thioureabenzyl(DOTA)trioxadiamine-1-(13"-Biotin-Asp-OH)trioxamine-5-Isothio-cyanato-Aminoisophthalate-rituximab.

40. A composition containing a medical agent according to any one of claims 32-39, wherein it further comprises physiologically acceptable additives, preferably an ammonium acetate solution.

41. Kit for extracorporeal elimination or at least reduction of the concentration of a non-tissue-bound therapeutic or diagnostic medical agent as defined in any one of claims 32-39 in the plasma or whole blood of a mammalian host, wherein said medical agent previously has been introduced to a mammalian host and kept therein a certain time in order to be concentrated to the specific tissues or cells by being attached thereto, said kit comprising

a) the anti-lymphoma antibody,

b) the reagent according to any one of claims 1-31 or the medical agent according to any one of claims 32-39, and

c) an extracorporeal device comprising an immobilised receptor on to which the affinity ligand adheres.

42. Use of a reagent according to any one of claims 1-31 for the production of a medicament for the treatment of lymphoma, preferably non-Hodgkin's lymphoma, wherein said reagent is conjugated to an anti-lymphoma antibody as defined in any one of claims 4-6.

43. Use of a medical agent according to any one of claims 32-39 for the production of a medicament for the treatment of lymphoma, preferably non-Hodgkin's lymphoma.

44. Method for treatment of lymphoma, preferably non-Hodgkin's lymphoma, wherein anti-lymphoma antibodies as defined in any one of claims 4-6 are administered to a patient with a need thereof, wherein complexes formed between said anti-lymphoma antibodies and leukocytes

having said one or more cell surface antigen(s) is/are then eliminated from the body of the patient, followed by administration of the medical agent according to any one of claims 32-39, optionally together with said anti-lymphoma antibodies as such, followed by extracorporeal elimination of the medical agent which has not been bound to the cell surface antigens on the lymphoma tumour cells.

ABSTRACT

A reagent for conjugation to an anti-lymphoma antibody is disclosed, as well as a medical agent containing said reagent, a kit containing said medical agent, use of said reagent and medical agent, and a method for treatment of lymphoma.

Election for publication: Figure 1

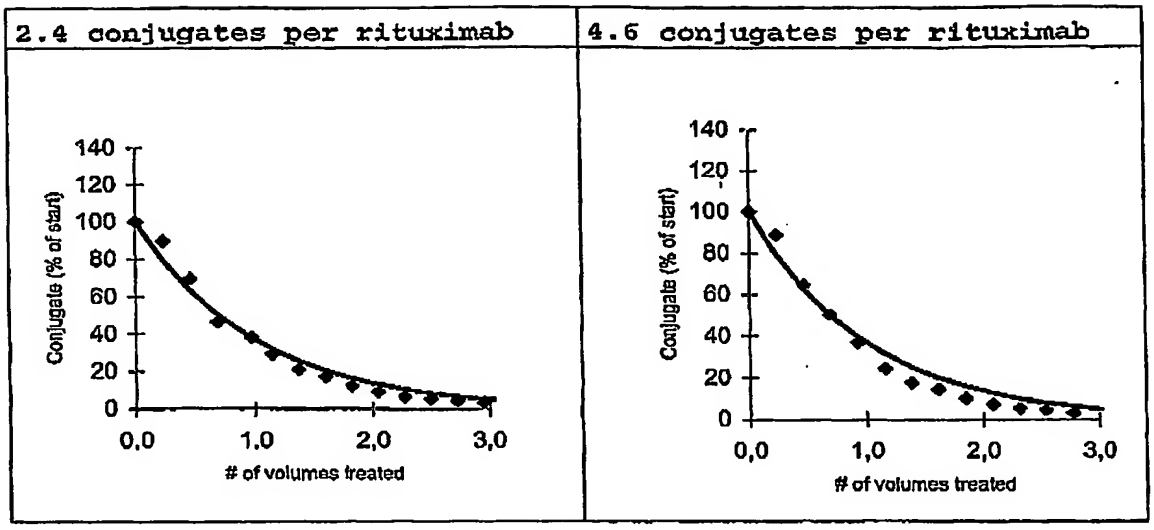
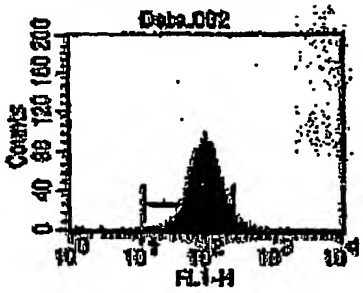
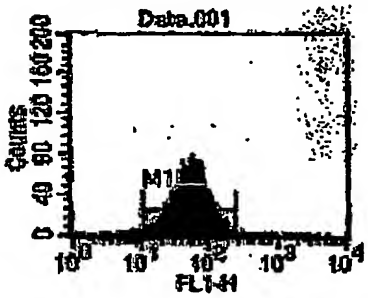


Figure 1: Depletion of 1033-rituximab conjugates during re-circulation through a miniaturised Mitradep®. The lines display the theoretically maximal possible depletion rate. As seen the depletion of 1033-rituximab is not different from the theoretical depletion line, i.e. all 1033-rituximab present in the solution passing through the device is removed.

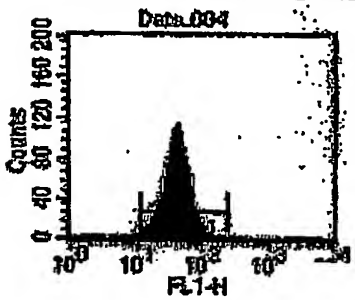
Biotin-Rituximab



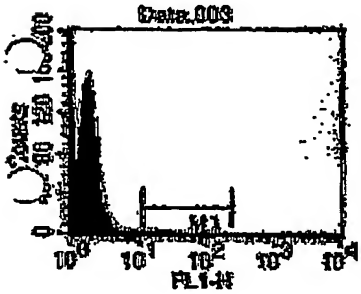
Biotin-mouse anti-CD20



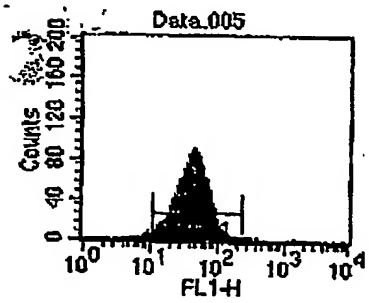
2.4 conjugate/Rituximab



Biotin-IgG



4.6 conjugate/Rituximab



PBS buffer

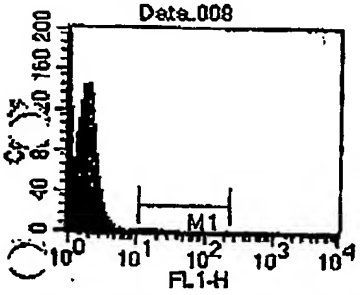


Figure 2: Flow cytometric assay of binding to the CD20-positive cell line

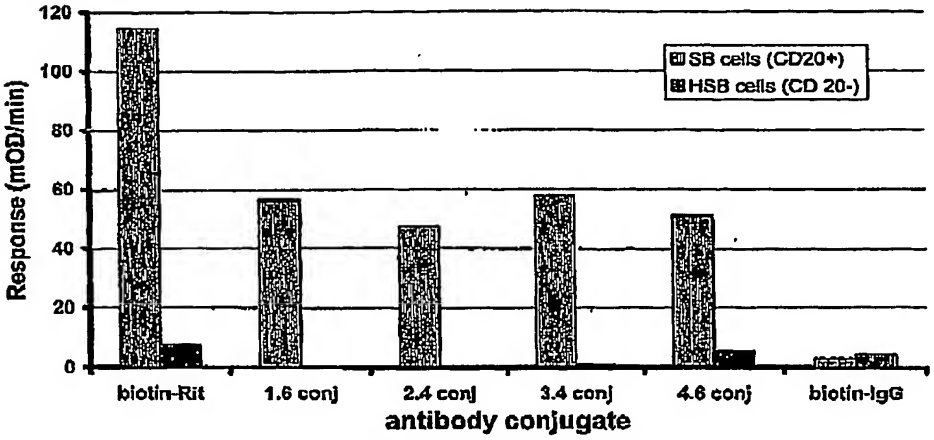


Figure 3: Binding of 1033-conjugates to a CD20+ (SB) and a CD20- (HSB) cell line. The binding is detected with an enzyme-labelled streptavidin.

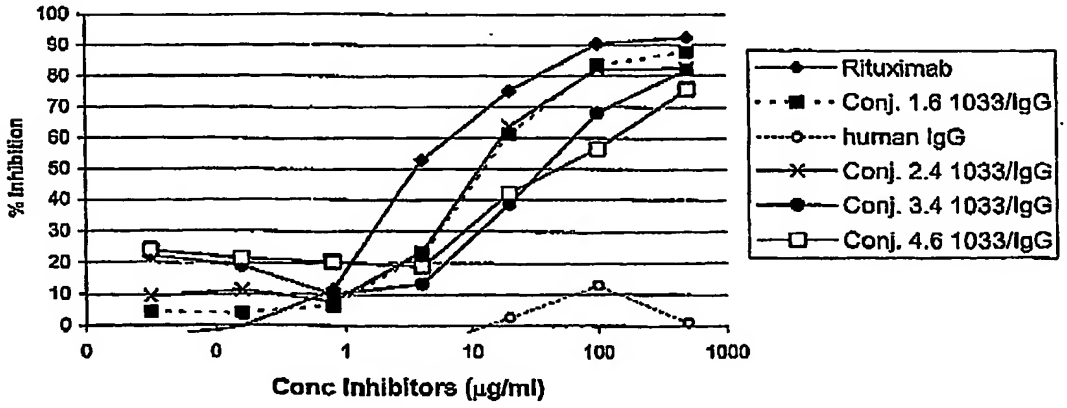


Figure 4: Competitive inhibition of ^{125}I-labelled rituximab binding to SB cells by cold rituximab and 1033-rituximab conjugates.

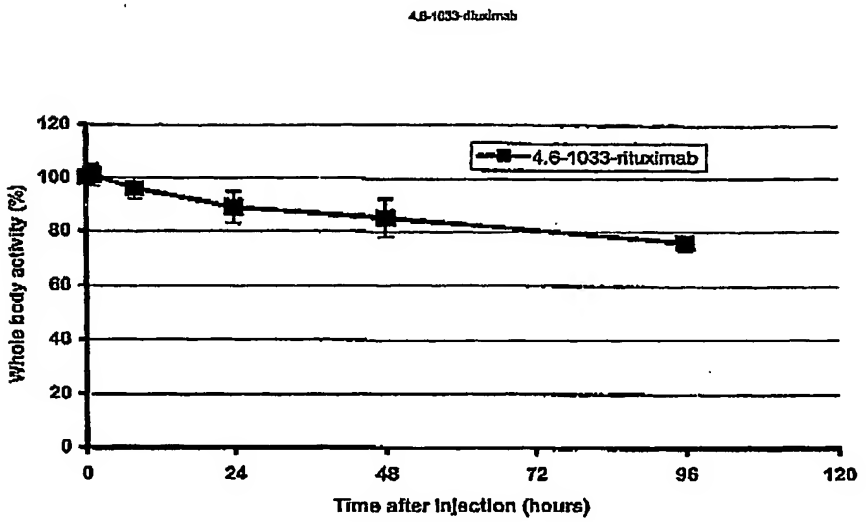


Figure 5: Whole body clearance of radioactivity in rats injected with ^{111}In-1033-rituximab antibody conjugates expressed as percentage ± std.dev. The data are corrected for radioactivity decay and background.

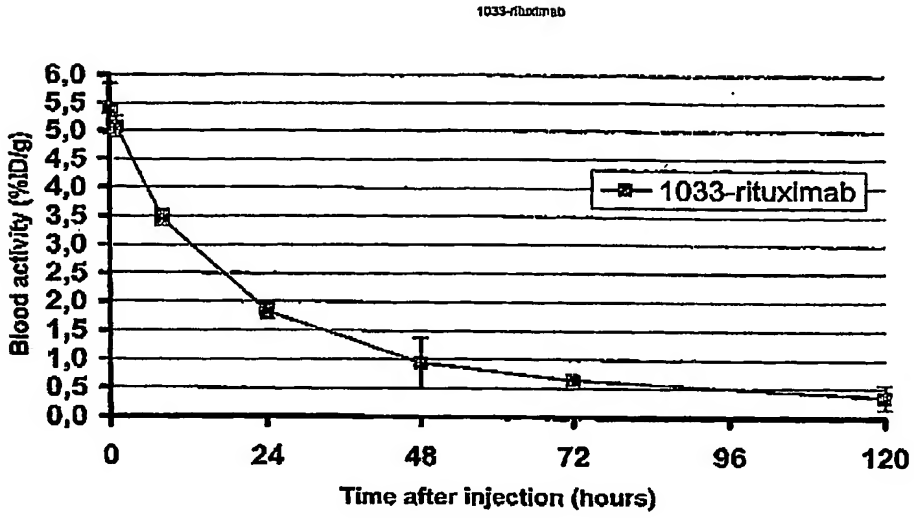


Figure 6: Blood clearance of ^{111}In-1033-rituximab antibody conjugates expressed as % injected dose/gram ± std.dev. The data are corrected for radioactivity decay.

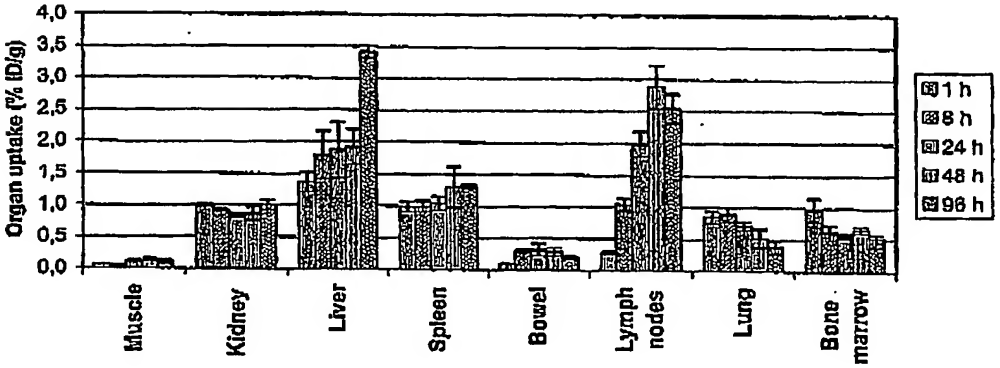


Figure 7: Biodistribution of ^{111}In-1033-rituximab (4.6 1033/IgG) in rats. The biodistribution is expressed as per cent of injected dose per gram tissue ± std.dev. The results are corrected for radiochemical decay.

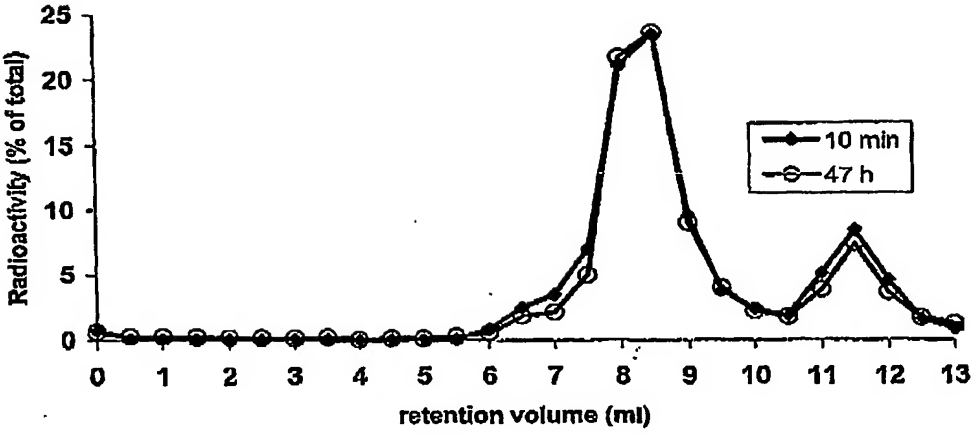


Figure 8: HPLC size exclusion separation of blood samples drawn from a rat injected with ^{111}In-1033-rituximab (4.6 1033/IgG).